*Biochimica et Biophysica Acta*, 722 (1983) 331–340
Elsevier Biomedical Press



BBA 41251

# LIPID ENRICHMENT OF THYLAKOID MEMBRANES

## I. USING SOYBEAN PHOSPHOLIPIDS

P.A. MILLNER, J.P. GROUZIS *, D.J. CHAPMAN and J. BARBER

*ARC Photosynthesis Research Group, Department of Pure and Applied Biology, Imperial College, London SW7 2BB (U.K.)*

(Received September 2nd, 1982)

*Key words: Lipid enrichment; Liposome; Freeze-fracture; (Pea thylakoid)*

**(1) Using asolectin (mixed soybean phospholipids) liposomes, extra lipid, with or without additional plastoquinone, has been introduced into isolated thylakoid membranes of pea chloroplasts. (2) Evidence for this lipid enrichment was obtained from freeze-fracture which indicated that a decrease in the numbers of EF and PF particles per unit area of membrane occurred with increasing lipid incorporation. The decrease was not due to loss of integral membrane polypeptides as judged by assay of cytochrome present or SDS-polyacrylamide gel electrophoresis of lipid-enriched membrane fractions. Moreover, the enrichment procedure did not lead to extraction of low molecular weight lipophilic membrane components or of thylakoid membrane lipids. (3) The introduction of phospholipids into the membrane affected steady-state electron transport. Inhibition of electron transport was observed when either water (Photosystem (PS) II + PS I) or duroquinol (PS I) was used as electron donor with methyl viologen as electron acceptor, and the degree of inhibition increased with higher enrichment levels. Introduction of exogenous plastoquinone with the additional lipid had little effect on whole-chain electron transport, but caused an increase in the 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB)-sensitive rate of PS I electron transport. The inhibition was also detected by flash-induced oxidation-reduction changes of cytochrome *f*.**

## Introduction

The concept that (PS I) and (PS II) are normally located in different regions of the thylakoid membrane of higher plant chloroplasts has gained increasing support in recent years [1–3]. Indications so far have been that PS II units are mainly present in the appressed membranes of the grana while PS I units are predominantly found in non-appressed thylakoids (i.e., in the exposed membranes of the granal and in the stromal lamellae). The existence of this lateral heterogeneity raises the problem of how PS II and PS I interact together both at the level of electron transport and energy transfer. One obvious answer, in the case of electron transport, is that plastoquinone functions as a long-range lipophilic lateral diffusing agent [2]. Such a notion is consistent with this step being rate limiting in electron transfer from water to a terminal acceptor on the reducing side of PS I. It seems unlikely that other redox components which are associated with integral membrane protein

* Present address: Laboratoire de Physiologie Vegetale, Universite des Sciences et Techniques du Languedoc, 34060 Montpellier Cedex, France.

Abbreviations: Chl, chlorophyll; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; PS, photosystem; DCMU, 3-(3,4-dichlorophenyl)-1,1 dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; PQ, plastoquinone; Mes, 4-morpholineethanesulphonic acid.

complexes could play roles in the long-range transfer of electrons from PS II to PS I because their diffusion rates would be too slow [2]. However, this may not apply to an extrinsic protein, like plastocyanin, which could in principle shuttle electrons over relatively large distances by diffusing along the membrane surface (Wraight, C.A., personal communication). As far as energy transfer is concerned, the regulation of spillover of excitons from PS II to PS I will also be dependent on the spatial relationship between the two photosystems under a particular set of conditions [3–5].

Clearly understanding the importance of the spatial relationships between the various integral complexes of the chloroplast thylakoid membrane is paramount to gaining a full knowledge of the electron-transfer processes of photosynthesis. The same type of argument also applies to respiratory electron transport and recently Hackenbrock and co-workers [6,7] have attacked this problem by varying the distance between various integral protein complexes by introducing additional lipids into the mitochondrial membrane. We have explored the possibility of this type of lipid enrichment with thylakoid membranes as have Siegel et al. [8] and the outcome of our initial studies are presented in this paper.

## Materials and Methods

Chloroplast thylakoids were prepared from peas as described previously [9] except that they were suspended as a stock in 0.33 M sorbitol, 25 mM KCl, 10 mM Hepes-KOH, pH 7.5, 5 mM $MgCl_2$ and 1 mg/ml bovine serum albumin (supension medium). Chloroplast stocks were kept on ice and all manipulations, except where indicated were carried out at 0–4°C.

Preparation of liposomes was commenced by rotary evaporating asolectin (100 mg L-α-phosphatidylcholine type IIS from Sigma) in 2 ml chloroform, onto the sides of a quickfit test tube. Where preparation of liposomes with plastoquinone incorporated were desired, 3.0 mg of $PQ_9$ (Hoffmann) were also dissolved in the chloroform prior to rotary evaporation and samples were maintained as far as possible in dim light. Approx. 0.2 ml of glass beads (0.5 mm diameter) were then added prior to addition of 1.5 ml of 10-fold diluted suspension medium. Liposomes were then formed by vortex mixing the mixture for 2 min prior to sonication under $N_2$ at 15–20°C for 45 min in a Pulsatron bath-type sonicator (Kerry Ultrasonics, Hitchin, Herts, U.K.).

To fuse liposomes with thylakoid membranes, chloroplasts (3 mg Chl) were diluted 5-fold with 10-fold diluted suspension medium and harvested by centrifugation at $5000 \times g$ for 10 min. The resulting pellet was then resuspended in 1.5 ml of freshly prepared liposomes and the pH rapidly adjusted to 6.0 by addition of 0.1 M HCl. After stirring the mixture at room temperature for 15 min it was overlayered onto a discontinuous sucrose gradient, consisting of 2.5 ml each of 0.4, 0.6, 0.8, 1.0 and 1.2 M sucrose in 10-fold diluted suspension medium and centrifuged for 1 h at $70\,000 \times g$ and 4°C using a Beckmann SW28.1 swing-out rotor. After centrifugation, bands forming at the gradient interfaces were removed and diluted with a large excess of 10-fold diluted suspension medium. The material collected from each band was then collected by centrifugation at $20\,000 \times g$ for 10 min and the pellets resuspended in small volumes (0.1–0.3 ml) of suspension medium.

Steady-state electron transport in the various membrane fractions derived by density gradient centrifugation was measured using a Hansatech oxygen electrode at 20°C. Saturating orange light (orange celluloid filter; cut-off approx. 540 nm) was provided by a Rank 2000 projector which was heat filtered through 1% $CuSO_4$ solution. Flash-induced cytochrome *f* redox changes ($A_{554} - A_{540}$) were measured with an Applied Photophysics flash spectrometer coupled to an E.G. & G. 546 C digital signal averager; 16 flashes were averaged for each wavelength. Electron-transport mediators were present as appropriate (see legends). For these optical measurements 60 μg Chl were present in 2 ml suspension medium, pH 7.5. Electron-transport mediators and inhibitors were present as appropriate (see legends) and when methanol was used as solvent its concentration was less than 1% (v/v).

Cytochrome *f* in lipid-enriched fractions was quantified from the ferricyanide (0.5 mM) minus hydroquinone (2.5 mM) reduced spectra [10] using 50 μg Chl in 1 ml of 50 mM Mes, pH 6.0, 1%

(v/v) Triton X-100: an extinction coefficient of 22 $mM \cdot cm^{-1}$ [10] at 554 nm was assumed. Measurements were performed on a Perkin-Elmer 557 double-beam spectrophotometer.

Freeze-fracture of lipid-enriched fractions was accomplished using a Balzer 301 freeze-fracture apparatus essentially as described in Ref. 11. Samples suspended in suspension medium were frozen in liquid freon, and fractured at −115°C and less than $2 \cdot 10^{-4}$ Pa pressure. Following cleavage, the fracture faces were shadowed with platinum and carbon and the replicas cleared as described in Ref. 11. The replicas were examined using a Phillips 301G electron microscope.

Total lipid and lipid class analyses of liposomes and of control and treated thylakoid membranes were performed as described in Refs. 12 and 13. Chlorophyll was determined by the method of Arnon [14].

Plastoquinone was determined using a Kontron high-performance liquid chromatography (HPLC) system equipped with a reverse phase (25 × 0.8 cm Spherisorb ODS 10) column. Chromatography of lipid samples prepared as described in Refs. 12 and 13 was carried out using a flow rate of 2.5 ml/min at 40°C with a detecting wave-length of 255 nm. Resolution of plastoquinone was achieved using a gradient of $H_2O$/methanol (7 : 93, v/v) to methanol/ethanol (1 : 1, v/v) as in Fig. 3 and a calibration curve was constructed utilizing $PQ_9$ supplied by Hoffman-La Roche.

All other chemicals were from Sigma or BDH and obtained at the highest purity available.

## Results

The time course for asolectin incorporation into pea chloroplast thylakoids is shown in Fig. 1 where it can be seen that the half-time for the lipid enrichment was approx. 4 min. Generally, about a 3-fold average enrichment in lipid could be obtained by this incubation procedure. When the lipid-enriched material was subjected to centrifugation on a discontinuous sucrose density gradient a number of fractions were obtained at the different gradient interfaces (Fig. 2), with the least dense fractions being the most enriched. Using this procedure it was possible to generate membranes which were enriched with lipid up to approx. 1500% compared with normal membranes (Table I; lipid/Chl). For assay of plastoquinone lipid extracts prepared from the gradient material were subjected to HPLC on a reverse phase column. A

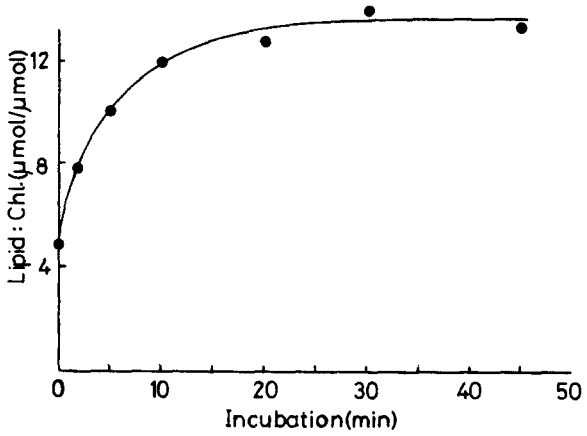


Fig. 1. Time course of asolectin uptake. After resuspending chloroplasts with asolectin liposomes and adjusting pH to 6.0, samples were taken at intervals up to 45 min and diluted ten-fold in suspension medium prior to immediate centrifugation at 20000 × *g* for 5 min. The resulting pellets were resuspended in suspension medium and analysed for lipid present. μmol lipid is calculated as μmol fatty acids/2 after treatment as described in Refs. 13 and 14.

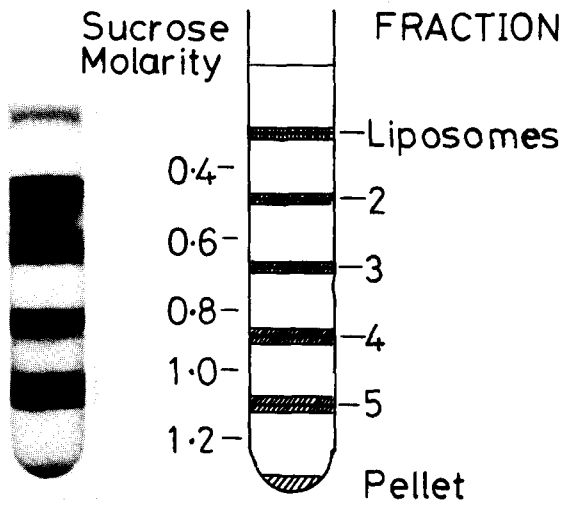


Fig. 2. Separation of lipid-enriched fractions on sucrose density gradients after incubation of thylakoids with asolectin liposomes as described in Materials and Methods. The discontinuous gradient consisted of 0.4, 0.6, 0.8, 1.0, 1.2 M sucrose in 10-fold diluted suspension medium, and the treated thylakoids were centrifuged for 1 h at 70000 × *g* and 4°C.

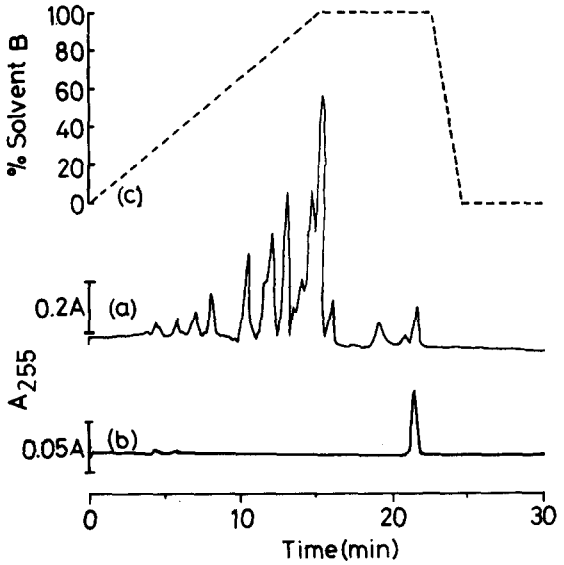


Fig. 3. HPLC of thylakoid lipid extract on Spherisorb ODS 10 (25×0.8 cm) reverse phase column. Flow rate = 2.5 ml/min; $T$ = 40°C; solvent A = methanol/water (93:7, v/v): solvent B = methanol/ethanol (1:1, v/v). Detecting wavelength was 255 nm. Traces are: (a) 35 μg total Chl injected, (b) 20 μg $PQ_9$ (Hoffman) injected, (c) gradient profile.

representative chromatogram is shown in Fig. 3 and it can be seen that the peak assigned to plastoquinone ($PQ_{ox}$) co-chromatographed with authentic plastoquinone ($PQ_9$). The same peak did not show absorbance at 450, 645 or 663 nm indicating a lack of any carotenoid or chlorophyll contamination. Table I shows that fractions derived from thylakoids enriched with asolectin alone showed a decreasing PQ/lipid ratio with increasing lipid incorporation; the uncombined liposomes remaining after centrifugation contained no detectable chlorophyll and little or no PQ indicating that PQ extraction from the chloroplast membranes had not occurred to any significant extent. In contrast, fractions derived from thylakoids enriched with asolectin liposomes containing plastoquinone showed a fairly constant PQ/lipid ratio which was equal to that for uncombined asolectin/PQ liposomes and about 350% of the control value. Analyses of liposomes recovered from the gradient after the enrichment procedure were also found to have a lipid class composition similar to that of the freshly prepared liposomes indicating that no thylakoid lipids had been extracted (see Table II).

The effect of introducing additional lipid on the ultrastructure of the thylakoid membrane has been investigated using the freeze-fracture procedure. Figs. 4 and 5 show EF and PF faces, respectively, of control (untreated) and lipid-enriched membranes. It is clearly evident from the micrographs that the particles shown in the fracture faces become more widely spaced as the lipid content of the membranes is increased (compare particularly

TABLE I

LIPID, CHLOROPHYLL AND PLASTOQUINONE CONTENT OF CONTROL AND LIPID ENRICHED THYLAKOIDS

Control and lipid enriched thylakoids were extracted with chloroform/methanol (2:1, v/v) and after removal of water as described in Refs. 13 and 14 the lipid extract was dissolved in chloroform. a, no chlorophyll detectable.

| Liposomes in incubation | Fraction | Lipid/Chl (mol/mol) | PQ/Chl (mol/mol) | PQ/lipid (mol/mol) (×10³) | Determinations |
|---|---|---|---|---|---|
| −PQ | Liposomes | a | a | 0.06 | 3 |
| | N2 | 43.6 | 0.031 | 0.66 | 2 |
| | N3 | 27.1 | 0.025 | 1.03 | 2 |
| | N4 | 12.7 | 0.027 | 2.13 | 3 |
| | N5 | 5.0 | 0.021 | 4.49 | 3 |
| +PQ | Liposomes | a | a | 36.7 | 2 |
| | P2 | 52.2 | 1.90 | 36.3 | 3 |
| | P3 | 24.2 | 0.90 | 37.1 | 2 |
| | P4 | 18.5 | 0.86 | 34.5 | 3 |
| | P5 | 6.4 | 0.17 | 26.4 | 3 |
| | Control | 2.9 | 0.029 | 10.2 | 3 |

Figs. 4A and D and 5A and D). The effect is quantified in Table III which gives the observed particle densities for the fracture faces in Figs. 4 and 5. Although these data confirm that lipid incorporation has occurred it should be noted that the extent of the enrichment was not proportional to the decrease in particle density; i.e., for example, a 10-fold enrichment did not lead to a 90% decrease in particles per unit area on the EF or PF faces. A possible explanation of this is the that the particles observed in untreated membranes undergo dissociation into particles of smaller diame-

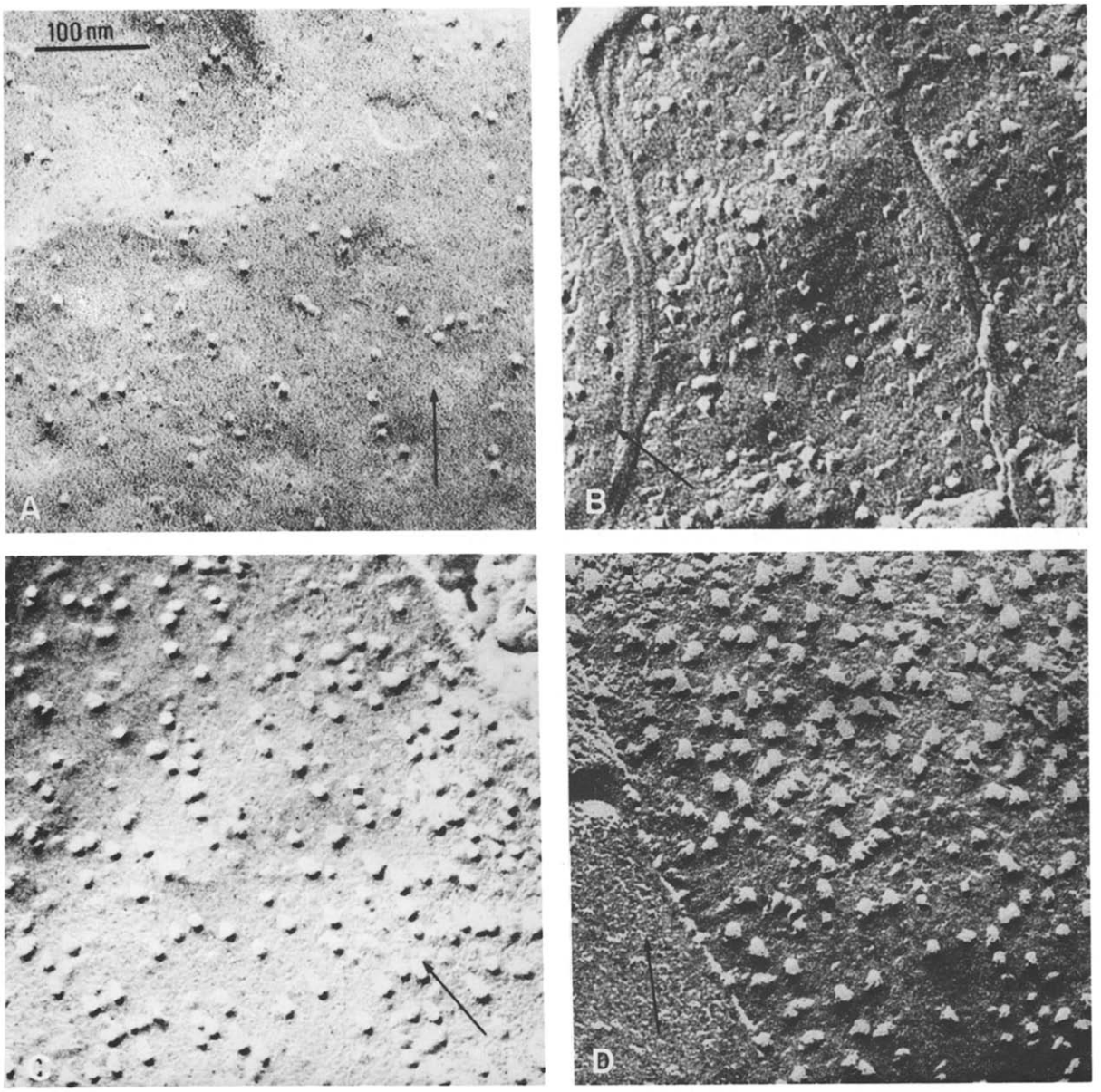


Fig. 4. EF fracture faces of lipid-enriched thylakoid fractions. Treated thylakoids were frozen without cryoprotectant and freeze-fractured in a Balzers 301 apparatus at $-115$°C and less than $2 \cdot 10^{-4}$ Pa pressure. Arrows show direction of shadowing and scale bar for A–D is shown in A. Percentage lipid enrichment over normal lipid content was: (A) 980; (B) 260; (C) 200; (D) 0 (control).

TABLE II

LIPID CLASS COMPOSITION (mol%) OF LIPOSOMES SAMPLED BEFORE AND AFTER THE ENRICHMENT PROCEDURE

Samples were taken of liposomes prior to lipid enrichment and from the top of the sucrose density gradient following enrichment. Lipid class analysis was carried out using thin-layer chromatography.

| | Liposomes | | Thylakoids (before enrichment) |
|---|---|---|---|
| | Before | After | |
| Monogalactosyl-diacylglycerol | 5.8 | 4.8 | 45.2 |
| Digalactosyl-diacylglycerol | 4.4 | 3.9 | 25.4 |
| Sulphoquinovosyl-diacylglycerol | 13.4 | 9.2 | 8.7 |
| Phosphatidylglycerol | 4.9 | 4.9 | 12.1 |
| Phosphatidylcholine | 40.1 | 42.5 | 4.7 |
| Other phospholipid classes | 31.5 | 34.6 | 4.0 |

ter which is indeed evident from comparison of Figs. 4A and D and 5A and D, and from the analyses of Siegel et al. [8].

To investigate the possibility that the lipid enrichment procedure could cause extraction of thylakoid membrane polypeptides, the cytochrome *f* content of the control and lipid-enriched membranes was measured. Table IV shows that there was a small decrease in the amount of hydroquinone reducible cytochrome *f*; but that this was

TABLE III

PARTICLE DENSITIES ON FREEZE-FRACTURE FACES OF LIPID ENRICHED CHLOROPLASTS

Data ± S.E. (number of fracture faces analysed). Percentage lipid enrichment relative to normal membranes was $B_2$, 980%; $B_3$, 260%; $B_4$, 200%. Control 0%. EF faces were taken as those with less than 1000 particles/$\mu m^2$ with the exception of $B_2$ where less then 500 particles/$\mu m^2$ was used.

| Fraction | Particle densities ($n/\mu m^2$) | |
|---|---|---|
| | EF faces | PF faces |
| $B_2$ | 388 ± 40 ($n = 8$) | 1030 ± 159 ($n = 5$) |
| $B_3$ | 624 ± 40 ($n = 5$) | 1788 ± 166 ($n = 7$) |
| $B_4$ | 700 ± 60 ($n = 5$) | 1651 ± 170 ($n = 7$) |
| Control | 803 ± 79 ($n = 5$) | 3449 ± 220 ($n = 6$) |

TABLE IV

CYTOCHROME *f* CONTENT OF CONTROL AND LIPID-ENRICHED THYLAKOIDS

Cytochrome *f* content was calculated from hydroquinone reduced minus ferricyanide oxidized spectra [10]. A baseline was drawn from 540 to 570 nm and $E_{mM} = 22$ at 554 nm was assumed for cytochrome *f*.

| Fraction | nmol cytochrome *f*/mg Chl |
|---|---|
| 2 | 1.37 |
| 3 | 1.46 |
| 4 | 1.45 |
| 5 | 1.46 |
| Control | 1.52 |

only 10–15% and the level of cytochrome *f* in all lipid-enriched fractions was fairly constant. Moreover, equal amounts of control and lipid-enriched material (as measured by chlorophyll content) were subjected to denaturing conditions and electrophoresed on SDS-polyacrylamide gradient gels (7–17% (w/v) acrylamide). No difference in the intensity of Coomassie blue-stainable polypeptide bands was found between control and lipid-enriched material (results not shown) which would again indicate that the enrichment procedure was not causing loss of membrane components.

In Table V typical results for the effect of asolectin enrichment on steady-state electron transport are shown. Using methyl viologen as an electron acceptor electron transport through both photosystems was profoundly inhibited, compared with control membranes in thylakoids enriched with or without plastoquinone present in the liposomes. Additionally, with increasing lipid enrichment this electron transport seemed to show a decreasing sensitivity to 5 $\mu$M DCMU added. When electron transport through PS I alone was monitored, using the cofactor duroquinol, which donates predominantly to the plastoquinone pool [15,16], similar rates were found for each fraction from thylakoids lipid enriched in the presence or absence of added plastoquinone. Generally, PS I rates were less inhibited than PS I + PS II rates and maintained up to 40–50% of the control electron-transport rate for the least enriched fraction. However, if the DBMIB-sensitive rates of electron transport in this system were considered then a

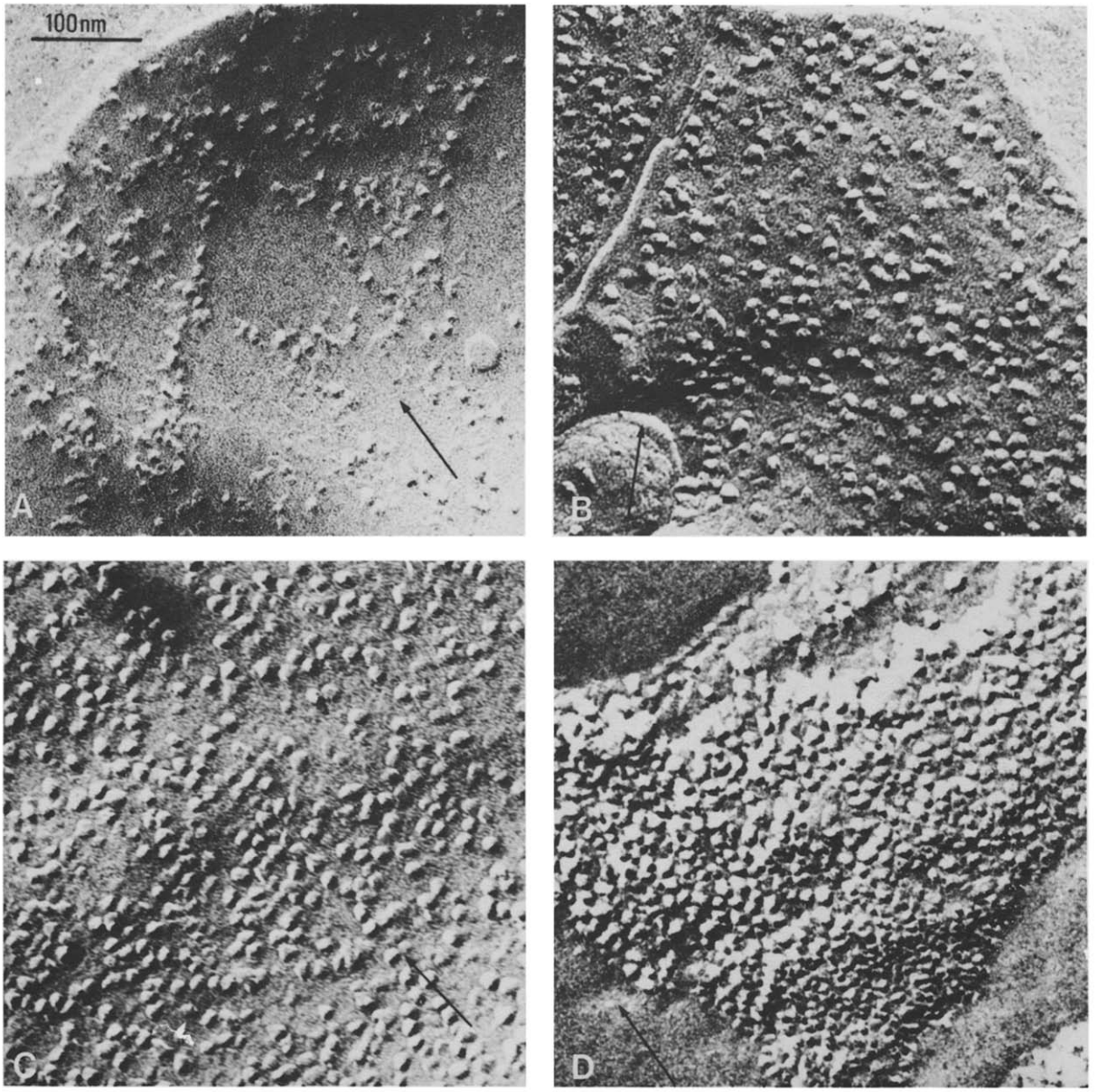


Fig. 5. PF fracture faces of lipid enriched thylakoid fractions. Details of preparation and degree of lipid enrichment were as for Fig. 4.

stimulation of electron-transport rates was found in fractions derived from thylakoids enriched with lipid and plastoquinone compared with the corresponding fractions from thylakoids enriched with lipid alone, with the exception of fraction 5. The latter fraction, which was the least enriched, showed no stimulation with plastoquinone present and frequently an inhibition as shown in Table V. However, the general trend was that the difference in rates with and without added plastoquinone was more pronounced in the most lipid-enriched fraction. The electron transport remaining, following DBMIB addition, probably represents lipid photo-oxidation, since use of white light to assay electron transport led to a large inhibitor-insensitive rate of oxygen uptake that was most pro-

TABLE V

PHOTOSYNTHETIC ELECTRON-TRANSPORT PARAMETERS OF CONTROL AND LIPID-ENRICHED (±PLASTOQUINONE) THYLAKOIDS

DBMIB-sensitive rate = rate(−DBMIB)−rate(+DBMIB). Rates are in μequiv./mg Chl per h. Results presented are for a typical experiment. Steady-state electron transport was assayed with membranes equivalent to 40 μg Chl in 2 ml suspension medium, pH 7.5. Gramicidin (2.5 μM), 0.5 mM $NaN_3$ and 0.5 mM methyl viologen were present initially and the following were added in sequence, with electron transport being monitored after each addition: 5 μM DCMU, 0.5 mM $DQH_2$ and 10 μM DBMIB.

| Liposomes in incubation | Lipid/Chl (mol/mol) | Fraction | Additions | | | | |
|---|---|---|---|---|---|---|---|
| | | | +Methyl viologen | +DCMU | +$DQH_2$ | +DBMIB | DBMIB-sensitive rate |
| −PQ | 44 | N2 | 54 | 43.5 | 189 | 125 | 64 |
| | 27 | N3 | 52 | 31.5 | 197 | 105 | 92 |
| | 13 | N4 | 46 | 22 | 261 | 190 | 70 |
| | 5 | N5 | 194 | 0 | 443 | 40 | 403 |
| +PQ | 52 | P2 | 56 | 39 | 232 | 131 | 101 |
| | 24 | P3 | 49 | 18 | 198 | 66 | 142 |
| | 19 | P4 | 46 | 22 | 208 | 118 | 90 |
| | 6 | P5 | 102 | 0 | 360 | 75 | 285 |
| | 3 | Control | 405 | 0 | 1012 | 78 | 934 |

nounced in the most enriched fractions.

On the basis that increasing the lipid content of the thylakoid membrane should reduce the effective concentrations of plastoquinone, the dark reduction of the flash-induced oxidation of cytochrome *f* was examined using reduced duroquinone as the electron donor to plastoquinone.

It can be seen from Table VI that both the amplitude of cytochrome *f* oxidation was reduced and the half-time for its re-reduction was increased with increasing lipid enrichment. Even in the least enriched fraction ($B_4$, 2-fold) these parameters were profoundly affected.

TABLE VI

EFFECT OF LIPID ENRICHMENT ON OXIDATION-REDUCTION KINETICS OF CYTOCHROME *f*

Flash-induced oxidation-reduction of cytochrome *f* in lipid-enriched thylakoids. Thylakoids were lipid treated with a sucrose density gradient comprised of 0.5, 0.75, 1.0, 1.25 and 1.5 M sucrose in 10-fold diluted suspension medium: the fractions $B_{2-4}$ were lipid-enriched to 1000, 260 and 200% of control, respectively. Absorbance changes at 554−540 nm were measured using 60 μg (Chl) of thylakoids in 2 ml suspension medium, pH 7.5. Cofactors present were: 0.5 mM, methyl viologen, 0.5 mM $NaN_3$, 5 μM DCMU, 0.5 mM duroquinol, 2.5 μM nigericin and 2.5 μM valinomycin. Traces shown are the average of 16 flashes for each sample. $\Delta A = \Delta I/I (\times 10^{-4})$.

| Fraction | $f_{ox}$ ($\Delta A$) | $f_{red}$ ($t_{1/2}$) (ms) |
|---|---|---|
| $B_2$ | 1.5 | > 200 |
| $B_3$ | 1.5 | > 200 |
| $B_4$ | 6.9 | 22 |
| Control | 11 | 15 |

## Discussion

By incubating chloroplasts with asolectin liposomes it is possible to introduce additional lipid into the thylakoid membrane producing an average lipid enrichment of about 300% with a half-time of approx. 4 min. Isolation of thylakoids with greater enrichments was achieved by using sucrose density centrifugation, since membranes with increasing amounts of lipid displayed decreasing buoyant densities. The maximum enrichment measured was approx. 1000%, although in principle it might be possible to separate out fractions with even greater lipid content by careful selection of the sucrose solution densities used in the gradient. Freeze-fracture studies confirmed that additional lipid was incorporated into the thylakoid membrane, in agreement with the recent work of Siegel et al. [8].

The decrease in the numbers of EF and PF face particles did not appear to be due to loss of protein as judged by separation of polypeptides on SDS-polyacrylamide gels (not shown) or by spectrophotometric estimation of cytochrome *f* content of the membranes.

Interestingly, the decrease was not to a level expected for the degree of lipid enrichment. For example, a 1000% increase in the lipid content compared to normal thylakoids did not result in a 90% decrease in particle number as expected. This is probably explained by the observation that both PF and EF particles appeared to be dissociating into smaller units, a phenomenon also observed by Siegel et al. [8]. Such an effect could be due to the introduction of additional phospholipids into the natural lipid matrix of the thylakoids which predominantly consists of uncharged mono- and digalactosyl lipids with only a small proportion of phospho- and sulpholipids [17,18]. Alternatively, the lipid/Chl ratio may have been an overestimation of the enrichment due to the adhesion of some non-incorporated asolectin vesicles. However, this was not evident from the freeze-fracture micrographs.

Estimation of the lipid, chlorophyll and plastoquinone level in the various sucrose gradient fractions indicated that when enrichment was carried out using asolectin alone the concentration of plastoquinone in the membrane fell with increasing lipid/Chl ratio. More importantly, little or no plastoquinone was found in the uncombined liposomes that remained above the lightest sucrose step after centrifugation, indicating that plastoquinone was not extracted from the thylakoid membranes in the enrichment procedure. Moreover, there appeared to be little or no other lipophilic components (carotenoids, other quinones, or thylakoid lipids) extracted by the asolectin liposomes during the enrichment procedure. Thus, the method presented, of introducing more lipid into the thylakoid membrane matrix, appears to lead to a spatial separation of membrane polypeptide complexes and also to a decrease in the concentration of membrane-soluble low molecular weight components without loss due to extraction by the non-incorporated liposomes. The functional effects of lipid enrichment appeared to be a profound inhibition of steady-state electron transport through both photosystems in all but the least lipid-enriched fraction, with little effect of the presence or absence of exogenous plastoquinone. However, if the DBMIB-sensitive electron transport through PS I alone was considered, utilizing the cofactor duroquinol which donates electrons to the plastoquinone pool [15,16], then a relative stimulation in this rate was observed in thylakoids lipid enriched in the presence of exogenous plastoquinone. The flash-induced oxidation-reduction kinetics of cytochrome *f* after incorporation of additional lipid into the membrane are in line with the observed effects on steady-state electron transport. Under the conditions used, we would expect oxidised cytochrome *f* to be reduced by plastoquinol, since again duroquinol was employed as the electron donor [15,16]. Both the amplitude of the initial cytochrome *f* absorbance change (indicating oxidation) and the half-time of its re-reduction were affected by the lipid enrichment.

These results in combination with the steady-state measurements indicate that the lipid enrichment procedure impaired normal electron flow. The stimulation found when plastoquinone was also incorporated would lend weight to its proposed role as a lipophilic diffusible electron carrier, as discussed by Anderson [2] and indicated by other indirect studies [19,20]. Lipid enrichment could additionally alter the spatial relationships between membrane components such as the cytochrome $b_6$-*f* complex [21] and the PS I reaction centre protein. Indeed, this possibility may account for the inhibition of cytochrome *f* oxidation and the failure to fully reconstitute PS I electron transport by incorporation of exogenous plastoquinone.

Overall, the work presented in this report has shown that it is possible to manipulate the lipid content of the thylakoid membrane, and also the level of low molecular weight membrane components. Such manipulation offers the chance to investigate more thoroughly the importance of the spatial relationship between integral protein components, and the role of diffusible lipophilic redox components such as plastoquinone, in the mechanisms of photosynthetic electron transport. Additionally, lipid enrichment procedures give the opportunity to study the role of lipids, both in terms of their chemical and physical properties as the support matrix for the functional proteins, and as

the phase for the diffusion of lipophilic molecules. The relevance of the type of work presented in this paper will become more evident when lipid enrichment is accomplished using lipids normally found in the thylakoids. Such work is underway and will be the subject of a further communication.

## Acknowledgements

Additionally, we thank the Agricultural Research Council and the Science Research Council for the financial help to undertake this work. The gift of $PQ_9$ from Hoffman La-Roche is acknowledged. J.P.G. wishes to thank The Royal Society for financial support from the European Science Exchange Programme.

## References

1 Andersson, B. and Anderson, J.M. (1980) Biochim. Biophys. Acta 593, 426–439
2 Anderson, J.M. (1981) FEBS Lett. 124, 1–10
3 Barber, J. (1980) FEBS Lett. 118, 1–10
4 Barber, J. (1980) Biochim. Biophys. Acta 594, 253–308
5 Barber, J. (1982) Annu. Rev. Plant Physiol. 33, 261–295
6 Hackenbrock, C.R. (1981) Trends Biochem. Sci. 6, 151–154
7 Schneider, E., Lemaster, J.J., Höchli, M. and Hackenbrock, C.R. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 442–446
8 Siegel, C.O., Jordan, A.E. and Miller, K.R. (1981) J. Cell Biol. 91, 113–125
9 Nakatani, H.Y. and Barber, J. (1980) Biochim. Biophys. Acta 591, 82–91
10 Anderson, J.M. (1982) FEBS Lett. 138, 62–66
11 Moor, H. and Muhlenthaler, K. (1963) J. Cell Biol. 17, 609–628
12 Khan, M.U. and Williams, J.P. (1977) J. Chromatogr. 140, 179–185
13 Williams, J.P. and Merrilees, P.A. (1970) Lipids 5, 367–370
14 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
15 White, C.C., Chain, R.K. and Malkin, R. (1978) Biochim. Biophys. Acta 502, 127–137
16 Izawa, S. and Pan, R.L. (1978) Biochem. Biophys. Res. Commun. 83, 1171–1177
17 Allen, C.F. and Good, P. (1971) Methods Enzymol. 23, 523–547
18 Harwood, J.L. (1980) in The Biochemistry of Plants, Vol. 4 (Stumpf, P.K., ed.), pp. 1–56, Academic Press, London
19 Yamamoto, Y., Ford, R.C. and Barber, J. (1981) Plant Physiol. 67, 1069–1072
20 Hirano, M., Satoh, K. and Katoh, S. (1981) Biochim. Biophys. Acta 635, 476–487
21 Hurt, K. and Hauska, G. (1981) Eur. J. Biochem. 117, 591–599